Chemical and antimicrobial studies of monoterpene: Citral

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ABSTRACT

6,7-Citral-epoxy derivative (a mixture of E and Z isomers with respect to the C2 = C3 double bond) could be react with DNA base producing a major adduct. The mixture of epoxides was condensed with 2 mol of cytosine to give the adduct through condensation between aldehyde and amino groups. Antifungal and antibacterial studies were carried out on citral and citral epoxide. Studies on the antifungal especially *Penicillium italicum* and *Rhizopus stolonifer* showed that citral and citral-epoxide have good antibacterial action. Antimicrobial studies of *P. italicum* and *R. stolonifer* explained also that citral and citral-epoxide have good antimicrobial activity. Citral epoxide shows high activity against the growth of bacteria methicillin resistant *Staphylococcus aureus* (MRSA) and fungi comparing by citral. The epoxide shows antibacterial activity more than the antibiotics nalidixic acid (NA) and ampicillin (AP) and nitrofurantoin (NI).The results revealed that these complexes are most effective against MRSA.

Keywords:
6,7-citral-epoxy derivative, Antifungal and antibacterial studies

1. INTRODUCTION

Cymbopogon citratus (DC) (Gramineae) is an herb worldwide known as lemongrass. The tea made from its leaves is popularly used as antispasmodic, analgesic, anti-inflammatory, antipyretic, diuretic and sedative [1]. The volatile oil obtained from fresh leaves of this plant is widely used by the perfumes, cosmetics industries and in traditional medicine for various purposes [2]. Citral is the major component of lemongrass oil which was extracted from its leaves, present at levels of, approximately, 65–85%. Citral (3,7-dimethyl-2,6-octadienal) geranial, geranylacetate and myrcene[2]. A number of dietary monoterpenes was shown to act effectively in chemoprevention and chemotherapy of different cancers in animal models, at cellular level, and in human clinical trials [4–6]. Unsaturated trepenes are capable of trapping activated oxygen species in vivo to give intermediate epoxides which can alkylate DNAs, proteins, and other bimolecular [7–12].

2. MATERIALS AND METHODS

2.1. Lemongrass leaves:
Scientific name: Cymbopogon citrates (*C. citratus*).Citral (1) was isolated by extraction of *C. citratus,* which was collected from Maddinah city (Saudi Arabia). Citral epoxide can be prepared in last work [13].

2.2. Test organisms
2.2.1. Fungi pathogenic :*Penicillium italicum* and *Rhizopus stolonifer* were obtained by the compilation of the Center for microbes (Mircen), Faculty of Agriculture, Ain Shams University – Arab Republic of Egypt. It was cultured on sabaroud dextrous agar media (Oxioid CM 41) at 25 OC.
2.2.2. Bacterial pathogenic :Methicillin resistant *Staphylococcus aureus* (MRSA) from Laboratory of Jeddah king Fahad Hospital in Saudi Arabia. It was cultured on Mueller Hinton media (Oxioid CM 41) at 37 OC

2.3. Standard antibiotic disc:
Nalidixic acid (NA) 30 µg, nitrofurantoin (NI) 300 µg, and ampicillin(AP) 25 µg, Mast Diagnostic Amiens, France.

2.4. The methods:
2.4.1. Alkylation of citral epoxide 2a with cytosine[12] .Mixture of citral epoxide 2a & a0 (0.00 µgm, 0.37 mol) and (0.22 gm, 0.002 mol) of cytosine was fused at 140 OC for half an hour to give gummy material which was treated with ethyl alcohol (2 ml) to give brown solid material. The residue was subjected to column chromatography on silica gel using petroleum ether (bp 60–80 _C)–diethyl ether (8:2) to isolate compounds 3. mp. 190 _C.
2.4.2. Citral-cytosine adduct [3].Colorless semisolid, C18H24N6O3 (M. wt. 372.00). IR (thin film): m–: 3413, 3175, 2954, 2857, 1634, 1462, 1149 cm_1. H,H NMR cosy (CDCl3): d: 0.9 (d, 3H, C8H3), 1.3 (br.s, 6H, 2 C9,10H3), 1.70 (Comp. pat., 2H, H-3), 2.3 (Comp. pat., 2H, H-4), 2.03 (Comp. pat., 1H, H-5), 4.23 (Comp. pat., 1H, H-2), 5.36 (d, 1H, H-6), 4.20 (d, 1H, H-7), 7.54 (d, 2H, 2H-50), 7.71 (d, 1H, H-6'), 7.72 (d, 1H, H-60), 9.71 (s, 1H, amide proton), 9.73 (s, 1H, amide proton) ppm. MS, m/z, 370 (M+-2H) (5%), 356 (M+_CH4) (36%), 336 (M+_C2H12) (50%), 278 (M+_C4H3N3O) (3%), 252 (M+_C5H2N3O) (7%), 168 (C10H18NO) (3%), 141 (C9H17O) (10%), 114 (C7H14O) (100%).
2.4.3. Biological activity of Lemongrass leaves oil (*C. citratus*): Lemongrass oil was tested for in vitro antimicrobial activity by using the agar-well diffusion method [24]. The antibacterial and antifungal activities of lemongrass oil on bacterium (MRSA) and fungi (*P.italicum* and *R. stolonifer*) (Table 1) .
2.4.4. Biological activity of citral and citral epoxide: Citral and its epoxide were tested against the fungal species *P. italicum* and *R. stolonifer*, and the bacterial species *S. aureus.*"
2.4.5. Antifungal activities :Diffusion method was used to evaluate the antifungal activities of the tested compounds [25]. The diameters of the fungal growth were measured after 2, 4 and 6 days (Table 2) .

2.5. Data analysis:
Analysis of data was carried out by student's t-test for comparing the means of experimental and control groups [28].

3. RESULTS

Citral, [2-(E), (Z)-3,7-dimethyl-octa-2,6-dienal] (1a,a), is a monoterpene aldehyde which is the major component of lemon grass oil extracted from C. citratus belonging to Gramineae [14–16] as a mixture of (2E)-and (2Z)-isomer at a ratio of 3:2, respectively. Thermal oxidation of citral using m-choroperbenzoic acid (mcpba) in chloroform at room temperature or photochemical oxidation with hydrogen peroxide using a sodium lamp, we obtained a mixture of (E & Z)-epoxides 2a & 2a0 in ca. 60% yield (in the ratio of 60:40 of E:Z configuration). No other products were observed. [13] (Scheme 1).
Epoxides could be reacted with DNA producing a major adduct [12]. Therefore, the mixture of epoxides 2a & a0 was condensed with 2 mol of cytosine to give the adduct 3 through condensation between aldehyde group and amino group in the first mole of cytosine to give intermediate X1, while the other molecule of cytosine was added to amino group on the epoxide ring and open it producing intermediate X2 which cyclized to give adduct 3 (Scheme 2). The 1H-NMRspectrum of 3 contained a doublet at d 0.90 ppmdue to protons of CH3 group and a singlet at d 1.30 due to protons of other two CH3 groups, and three doublets at d 7.54, 7.71 and 7.72 ppm due to protons of two methylene groups in cytosine ring. Mass spectrum showed the molecular ion peak (M+_2H) at m/z 370.
The antimicrobial activity against on *Staphylococcus aureus* (MRSA), *Penicillium italicum* and *Rhizopus stolonifer* was also studied ,and the results obtained showed the important antimicrobial activity of Lemongrass oil (Table 1). The antifungal result (Table 2) showed that the growth of *P. italicum* and *R. stolonifer* on the solid media was reduced in the presence of Citral and its epoxide on fungi. Studies on the antifungal especially *P. italicum* and *R. stolonifer* activity of two essential oil components (citral and menthol) were reported [17] .The data in (Table 3,4) revealed that there was a significant decrease in the growth of (MRSA) on liquid media ,when(MRSA) were treated by Citral epoxide than Citral . The same result has been explained in the diffusion method which showed inhibition zones around the antibiotic disc(Fig. 2) [18]. Citral epoxide showed more activity than that of the Citral against bacteria .The activity of epoxide has been compared with the activity of standard antibiotics Nalidixic acid (NA)and Ampicillin(AP) ,but its showed same activity of Nitrofurantoin (NI), . The results revealed that these complexes are most effective against MRSA.

TABLE (1) Diameter of inhibition zone of the lemongrass oil againt *Staphellococcus areus* , *Penicillium italicum* and *Rhizopus stolonifer.*

Test strains	Diameter of inhibition zone(mm) lemongrass oil(µL)
Staphylococcus aureus	3.5
Penicillium italicum	2.6
Rhizopus stolonifer	1.8

TABLE (2) : Effect of Various Concentrations of Citral and Citral epoxide on the Radial Growth of *Pencillium italicum* and *Rhizopus stolonifer* Grown on Solid Media.(mm disc; Mean of Replicates SE).

Treatment	Concentration	Fungi pathogenic	Days 2	Days 4	Days 6
Control	0.0	*P.italicum*	7.07 ± 0.23	8.23 ± 0.15	9.00 ± 0.00
		R. stolonifer	4.37 ± 0.19	5.27 ± 0.18	9.00 ± 0.00
Citral	0.5	*P.italicum*	6.70 ± 0.15(ns)	7.77 ± 0.15*	8.03 ± 0.09*
		R. stolonifer	2.27 ± 0.15**	6.17 ± 0.09**	6.67 ± 0.28**
	1.0	*P.italicum*	2.90 ± 0.21**	6.53 ± 0.03**	7.50 ± 0.29**
		R. stolonifer	1.33 ± 0.03**	4.40 ± 0.12**	5.17 ± 0.09**
Citral epoxide	0.5	*P.italicum*	2.23 ± 0.15**	4.60 ± 0.21**	4.93 ± 0.35**
		R. stolonifer	1.93 ± 0.23**	6.37 ± 0.27**	8.80 ± 0.12 (ns)
	1.0	*P.italicum*	1.00 ± 0.06**	1.30 ± 0.12**	1.80 ± 0.29**
		R. stolonifer	1.50 ± 0.29**	2.83 ± 0.17**	3.30 ± 0.06**

**Significant at P ≤ 0.01 (ns) Non significant at P ≤ 0.05 *Significant at P ≤ 0.05

TABLE (3) : Effect of Various Concentrations of Citral and Citral epoxide on the growth rates of *Staphelococcus areuse* after 24h .

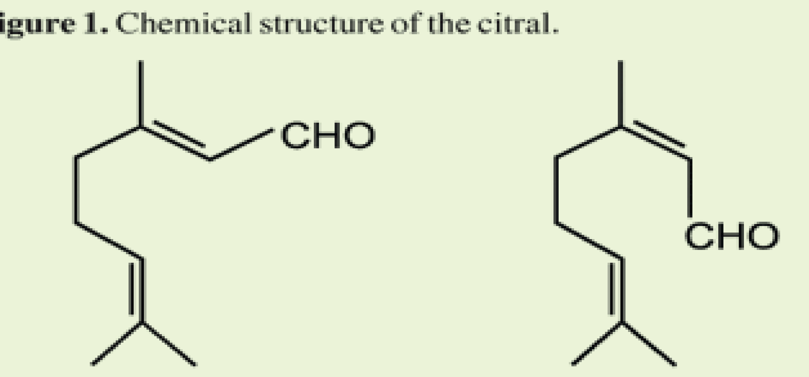
Treatment	Concentration	*Staphelococcus areuse*
Control	0.0	185.00 ± 2.89
Citral	0.5	153.33 ± 3.33**
	1.0	110.33 ± 0.88**
Citral epoxide	0.5	123.33 ± 0.88**
	1.0	54.00 ± 3.06**

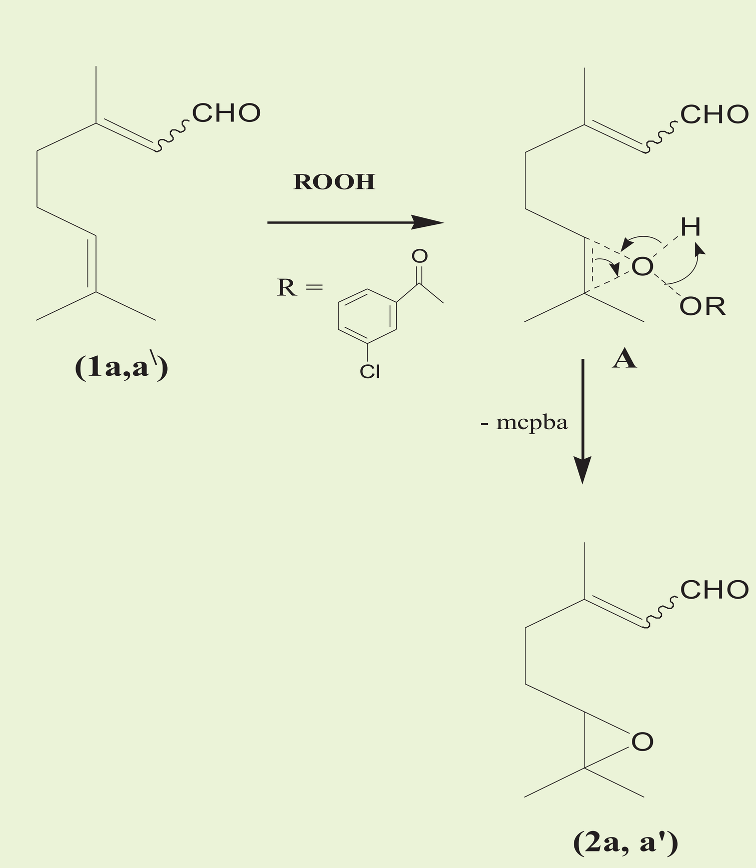
*Significant at P ≤ 0.01

TABLE (4) : Antibacterial activity data of Citral ,Citral epoxide and some known antibiotics

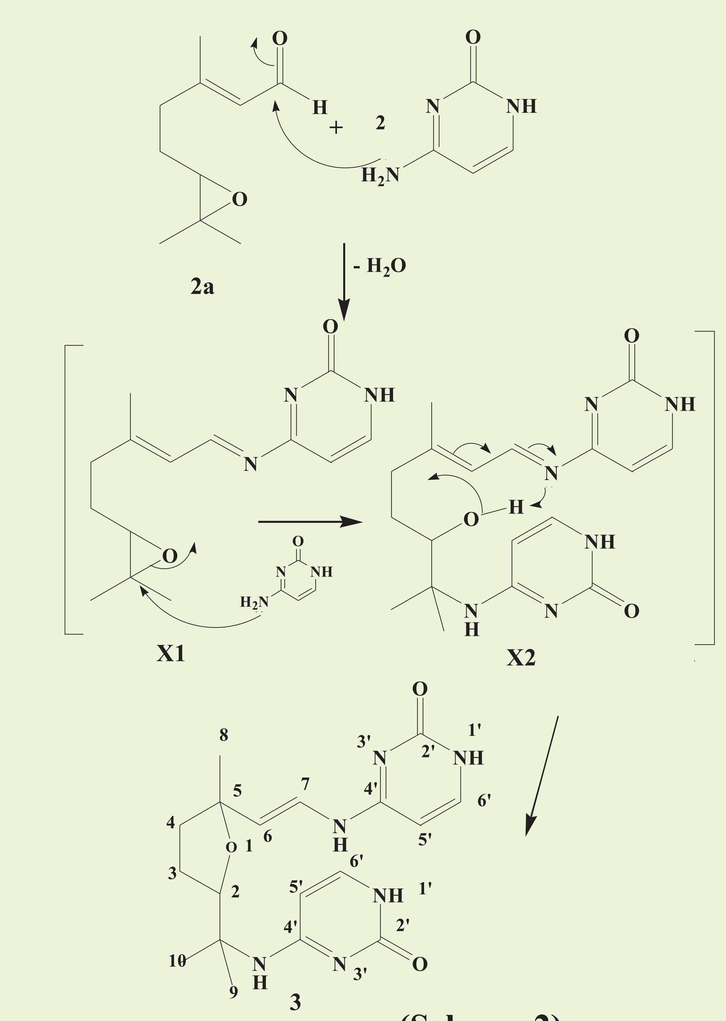
ANTIBIOTIC	*Staphelococcus areuse*(MRSA)
Nalidic acid (NA)	++
Ampicillin(AP)	R
Nitrofurantoin (NI)	+++
Augmantin(Ag)	++
Citral	+
Citral epoxide	+++

Figure 1. Chemical structure of the citral.





(Scheme 1)



(Scheme 2)

Fig.(2):Effect of Citral epoxide on radial growth of fungi grown on the solid media (a)control (b) *Penicillium italicum* (c) *Rhizopus stolonifer.*

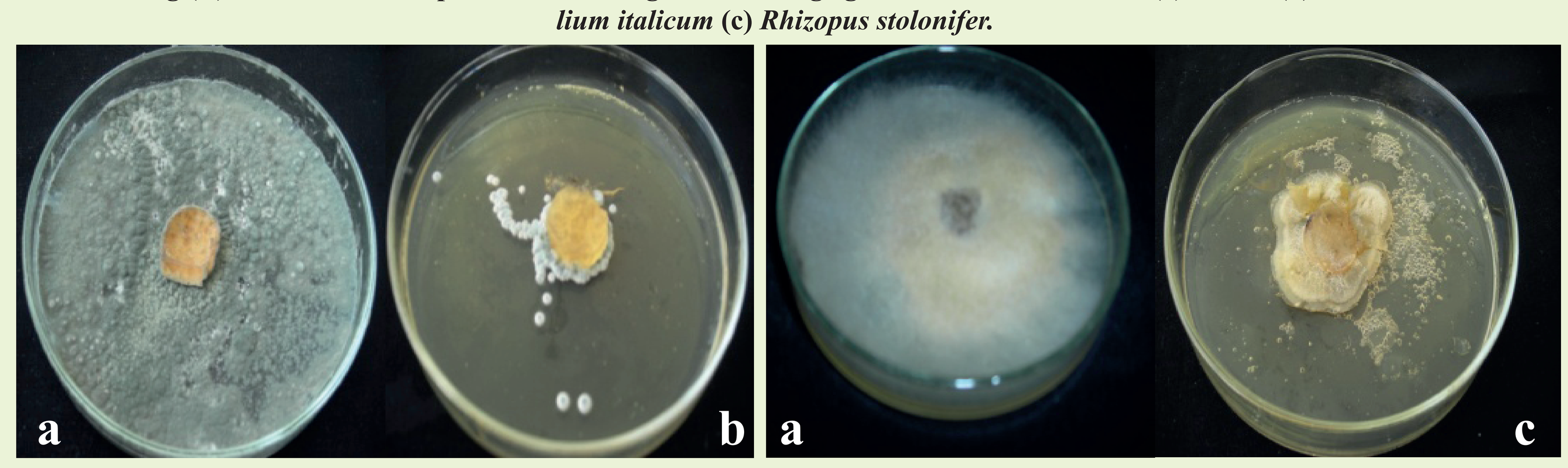
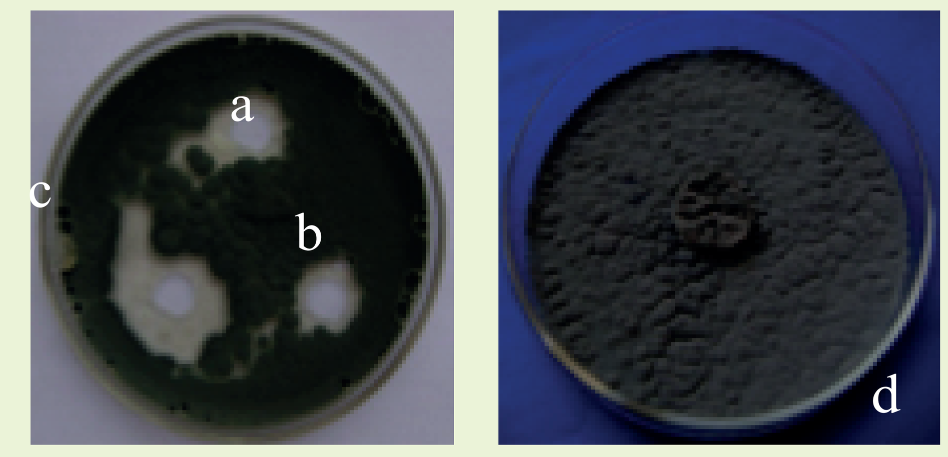


Fig.(3):Effect of lemon gress leaves oil (a) Citral (b) Citral epoxide (c) on the radial growth of *P. italicum* grown on the solid media(d) control.



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